Membrane Interface for On-Line Supercritical Fluid Extraction/ Flow Injection Analysis

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A simple interface between a supercritical carbon dioxide extractor and an on-line liquid-phase analytical system such as flow injection analysis (FIA) or HPLC is described. Previous approaches to on-line coupling of SFE and HPLC utilized either a separate sorbent bed or the HPLC column itself to separate the analyte from CO_2 in the extract sample. The former approach eliminates interference from CO_2, but requires development of trapping and elution conditions for each analyte. The latter approach suffers from interference by the large volume of CO_2 introduced into the HPLC system. The interface described here uses a membrane phase separator to remove CO_2 from the extract sample without the need for HPLC columns, etc., eliminating interferences while quantitatively transferring solutes of all types to the analytical system. The determination of chloramphenicol and penicillin G solubility in supercritical carbon dioxide with analysis time of 2 min is demonstrated with an on-line SFE/FIA system utilizing this interface.

INTRODUCTION

Supercritical fluid extraction (SFE) is a promising technique for isolation of analytes from complex matrices. Among the advantages of SFE over conventional liquid extraction are the use of environmentally safe solvents (e.g., carbon dioxide), and the ability to "tune" solvent strength through adjustment of extraction temperature and pressure. We are particularly interested in the use of SFE with unmodified CO_2 for extraction of antibiotics from animal tissue. In order to assess the feasibility of SFE for isolation of these compounds, their solubility in supercritical CO_2 must be determined over a range temperature and pressure conditions. Due to the polar nature and thermal lability of these compounds, extracts are typically analyzed by HPLC. One limitation of SFE is the difficulty in interfacing to liquid-phase analytical techniques such as HPLC and FIA (flow injection analysis).[1,2] Both off-line interfaces which vent the extract sample through a restrictor into a cold trap[3], a liquid solvent,[4] or a solid sorbent[5] and on-line interfaces using sorbent traps[6] or simply direct injection of the extract sample[7-9] have been described. None of these approaches is ideal. Aerosol formation and precipitation of analyte in the restrictor[1,2] are common problems with off-line interfaces and can result in poor analyte recovery or failure of the apparatus. Dilution of analyte is a significant problem with solvent trapping, and use of solid sorbents in on-line or off-line modes requires testing to determine conditions for quantitative trapping and efficient elution of each analyte. Care must also be taken to control the nature of the sample, since sample matrix components such as water can severely affect analyte trapping efficiency.[5] Direct injection of supercritical extracts into an HPLC or FIA system affords a very simple approach to SFE/HPLC interfacing, but the large volume of gas produced as the extraction solvent decompresses can result in significant interference with analyte separation and detection.[7,9] Both bubbles from undissolved gas and refractive index disturbances due to dissolved CO_2 have been observed. This problem is especially pronounced in reversed-phase HPLC with polar solvents (e.g., methanol) and appears to be due to the high solubility of CO_2 in these solvents. Retention times of analytes may be increased to avoid interference from dissolved CO_2, but this tactic results in excessively long analyses and cannot prevent disruption by bubbles which become trapped in the detector. Damage to the column bed by the large pressure pulse which occurs when the high-pressure fluid is injected and shifts in eluent pH as CO_2 dissolves to form carbonic acid may also be encountered with direct injection.

We have found that a membrane phase separator in conjunction with a high-pressure sampling valve and a short capillary restrictor provided a simple, rapid, general purpose method for on-line coupling of SFE and liquid-phase analytical systems. The properties of this interface and its use in the SFE/FIA mode for measuring the solubility of two pharmaceuticals in supercritical carbon dioxide are described here.

EXPERIMENTAL SECTION

Reagents and Materials. SFC grade carbon dioxide with helium headspace (Scott Specialty Gases, Plumsteadville, PA), methanol (Burdick and Jackson, Muskegon, MI), chloramphenicol (reference standard, gift of Dr. Daniel P. Schwartz, USDA, Philadelphia, PA), penicillin G (benzylpenicillin, procaine salt, Sigma, St. Louis, MO), microporous polypropylene membrane (Celgard 2400, Celanese Plastics Co., Greer, SC), and porous polypropylene sheet (Fritware, 72-μm pore size, $^1/_{16}$-in. thick, Bel-Art, Pequannock, NJ) were used as received. The 50-μm microporous fluoropolymer membrane (PTFE Thread Seal tape) was purchased locally. HPLC grade water was used to prepare the 30% methanol mobile-phase solvent.

Apparatus. The gas/liquid phase separator was locally constructed, with a liquid flow channel 0.1 mm thick and an

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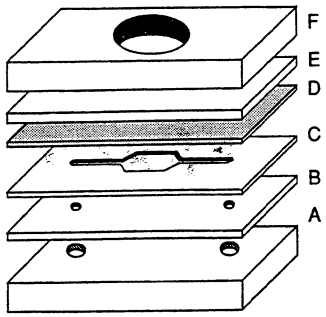

Figure 1. Membrane phase separator: (A) lower PMA block; (B) fluoropolymer sheet; (C) polyethylene fluid channel; (D) PTFE membrane; (E) porous polypropylene membrane support; (F) upper PMA block.

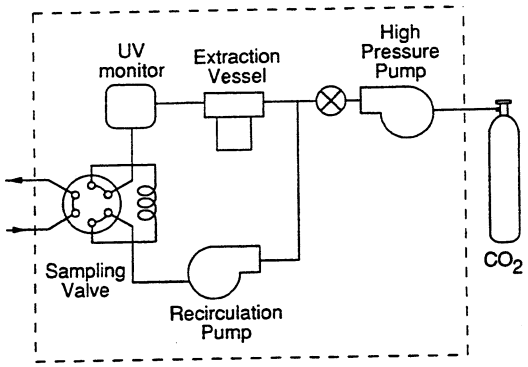

Figure 2. Simplified block diagram of the SPA extractor.

active membrane area of ~2 cm². As shown in Figure 1, the separator was formed by clamping a 0.35-mm fluoropolymer sheet containing holes for liquid inlet and outlet (B), a 0.2-mm polyethylene sheet containing the liquid flow channel (C), a 50-μm PTFE membrane (D), and a 1.6-mm porous polypropylene membrane support sheet (E) between two poly(methyl methacrylate) (PMA) blocks (A, F). The PMA blocks were 7.5 cm long, 5 cm wide, and 0.85 cm thick. Eight bolts passed through holes (omitted for clarity) around the periphery of the separator to provide clamping pressure. Threaded connections for standard 1/4-28 low-pressure chromatography fittings were made in the lower PMA block, and the upper block had a 20-mm hole through the center to allow gas to escape. Total fluid volume in the separator was approximately 50 μL, and all wetted surfaces were fluoropolymer or polyethylene.

Supercritical fluid extractions were conducted in a SPA extractor (sample preparation apparatus, LDC Analytical, Riviera Beach, FL). As shown in Figure 2, the SPA[9,10] is a recirculating extractor in which the supercritical solvent is pumped continuously through a closed loop consisting of the extraction vessel, on-line UV absorbance monitor, sampling valve, and recirculating pump. For the purposes of this work, the SPA may simply be regarded as a device which produces a solution of the analyte in supercritical CO_2 at a given temperature and pressure. By use of a large mass of sample and a sufficiently long recirculation period, the solution can be saturated with the analyte. Samples (10 μL) of this solution were withdrawn from the recirculation loop via an air-actuated fixed-loop sampling valve and injected into an external fluid stream for analysis.

Direct-injection SFE/HPLC experiments were performed with a system consisting of an HPLC pump (Model 114M, Beckman Instruments, Inc., Fullerton, CA), an injection valve (Model 7010, Rheodyne, Inc., Cotati, CA), the SPA sampling valve, an HPLC column (LC-18, 4.6 × 250 mm, Supelco, Inc., Bellefonte, PA), a UV detector (Model 785A, Applied Biosystems, Inc., Ramsey, NJ), and an integrator (HP 3396A, Hewlett Packard, Avondale, PA). The mobile-phase flow rate was 0.8 mL/min, the mobile phase was 30% methanol, and absorbance was monitored at 254 nm.

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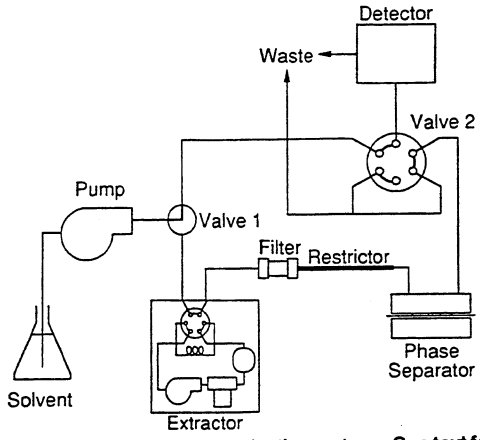

Figure 3. SFE/FIA solubility determination system. See text for details.

SFE/FIA experiments were performed with the sampling interface and analysis system shown in Figure 3. This consisted of an HPLC pump (Model 2350, Isco Inc., Lincoln, NE), a high-pressure stream selection valve (valve 1, Model 7060, Rheodyne, Inc., Cotati, CA), the sampling valve of the SPA extractor (10-μL loop), a 2-μm in-line HPLC filter (Upchurch, Oak Harbor, WA), a 50 μm i.d. × 15 cm long fused-silica capillary restrictor (Polymicro Technologies, Phoenix, AZ), a membrane phase separator, a low-pressure six-port valve (valve 2, Cheminert Model B60SV, Valco Instruments Co., Inc., Houston, TX), a diode array UV detector (Model 1000S, Applied Biosystems, Inc.), and an integrator (HP 3396A, Hewlett Packard). Stainless steel tubing with inside diameter of 0.020 in. was used between the pump and the sampling valve, while tubing with an inside diameter of 0.010 in. was used between the sampling valve and the filter. All other connections were made with fluoropolymer tubing having 0.5-mm i.d. Valve 2 had integral 1.5 mm i.d. × 50 mm long fluoropolymer tubing sections connected to each port, yielding a swept volume of ~200 μL. The total dead volume between the sample injection valve and detector, including valve 2, was ~300 μL.

Procedure. The extraction vessel was filled with ~100 mg of analyte dispersed on ~1 mL of 0.3-mm glass beads. The extractor was charged with carbon dioxide and adjusted to the desired temperature and pressure as described previously.[10] Extraction was continued (recirculation pump on) for ~30 min at each pressure in order to obtain a saturated solution of analyte. A sample of the extract was removed for HPLC or FIA analysis by actuating the SPA sampling valve. The sampling valve was then flushed with CO_2 and switched back into the extraction loop. The extraction pressure was raised by pumping additional CO_2 into the recirculation loop, the system was equilibrated at the new pressure, and the sampling cycle was repeated.

For direct-injection SFE/HPLC, a 10-μL sample of extract was injected into the HPLC eluent stream by actuating the SPA sampling valve while the HPLC pump was operating. Chromatograms of samples dissolved in mobile phase were obtained by using the HPLC injection valve. For on-line SFE/FIA, the following procedure was used. During extraction/equilibration, valves 1 and 2 were in the positions shown in Figure 3, and carrier liquid (30% methanol) was pumped at 0.5 mL/min directly to the detector, bypassing the extractor and phase separator. To initiate sample withdrawal and analysis, valve 2 was switched to connect the phase separator to the detector, and then the SPA sampling valve was actuated. After allowing ~60 s for expansion of the carbon dioxide through the restrictor and phase separator, valve 1 was switched and solvent was pumped through the sampling valve, carrying the sample through the phase separator and on to the detector, where the absorbance was measured and recorded. Chloramphenicol absorbance was measured at 273 nm, penicillin G at 290 nm. Calibration of the SFE/FIA system was performed by substituting an HPLC injection valve for the SPA sampling valve and injecting 10-μL aliquots of standard methanolic drug solutions into the carrier stream. A calibration curve was prepared by plotting peak area vs concentration injected and used to quantitate the amounts of drug in the supercritical extracts.

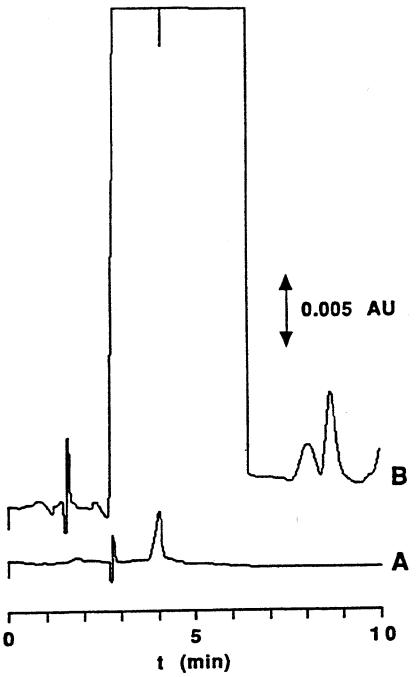

Figure 4. Direct-injection SFE/HPLC: (A, bottom) chromatogram of penicillin G dissolved in mobile phase at 0.04 mg/mL (conventional injection); (B, top) chromatogram of directly injected supercritical CO_2 (no analyte present) at 40 °C and 1000 psi. Conditions: mobile phase, 70:30 water/methanol; flow rate, 0.8 mL/min; injection volume, 10 μL; detection, UV absorbance at 254 nm.

RESULTS AND DISCUSSION

Initial experiments with direct injection of supercritical CO_2 extracts into a reversed-phase HPLC system resulted in large "noise" peaks eluting near the column void volume as well as large baseline excursions which completely obscured the analyte peak, as shown in Figure 4. These interferences were attributed to the CO_2 injected into the system, and a means of removing CO_2 on-line prior to injection into the HPLC was sought. Because the 10-μL volume of supercritical CO_2 initially injected into the liquid stream expanded to several milliliters upon decompression, the CO_2 removal device required a gas-handling capacity on the order of 5 mL. An "inverted-T" bubble trap similar to those used on HPLC solvent inlet lines was tested for this purpose, but the large dead volume of the trap resulted in extensive broadening and dilution of the analyte plug. This approach was therefore abandoned, and the use of a membrane phase separator was investigated.

Membrane phase separators have been widely used in FIA for liquid/liquid extraction,[11] in SFE/SFC of aqueous samples for the separation of water and supercritical CO_2,[12] and in HPLC for removal of dissolved gases in eluents.[13] A planar membrane phase separator of conventional design was constructed and tested in a low-pressure flow system consisting of a pump, loop injection valve, and phase separator. Air bubbles were introduced into the carrier liquid by manually filling the sample loop with a mixture of air bubbles and carrier. The carrier flow rate, carrier composition, and proportion of air bubbles to liquid were varied. Both polypropylene and PTFE membranes were effective at removing air bubbles with water as the carrier liquid, but the polypropylene membrane leaked when methanol/water mixtures were used. The PTFE membrane was usable with

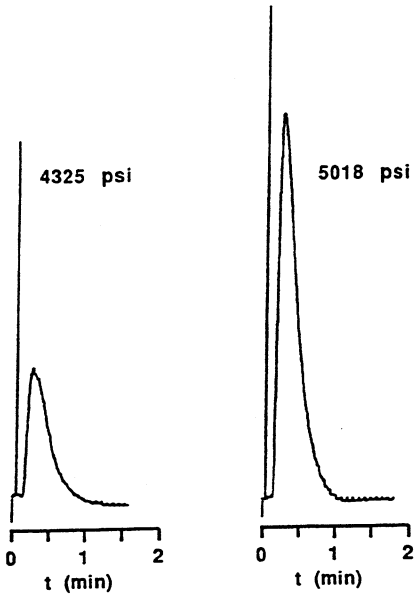

Figure 5. FIA analysis of penicillin G in supercritical CO_2 at 40 °C. Conditions: solvent, 70:30 water/methanol; flow rate, 0.5 mL/min; detection, UV absorbance at 290 nm. Pressure as indicated.

carriers containing up to 30% methanol or 40% acetonitrile, although the lifetime of the membrane varied with the carrier composition and pressure history. With methanol concentrations below 20% and membrane pressure differentials below 1 atm, membranes lasted at least 1 week. With methanol concentrations between 20% and 30%, membrane lifetime was reduced to 2–4 h. This could be extended to several days by limiting solvent flow through the phase separator to periods when sample was actually being analyzed. This constraint on carrier liquid composition in SFE/FIA is not expected to impede use of the interface with compounds which would typically be analyzed by reversed-phase HPLC. We have not tested the interface with extraction solvents other than carbon dioxide, but mixtures of carbon dioxide and organic modifiers at levels typically used (5–10%) are not expected to present difficulties.

The phase separator was incorporated into a flow injection analysis system and coupled to the extractor sampling valve with a capillary restrictor (Figure 3). The restrictor dimensions were adjusted by trial and error to provide the necessary pressure drop between the expanding extract sample and the phase separator. Care was taken to avoid any source of back pressure downstream of the phase separator which could result in an excessive pressure differential across the membrane. An in-line filter was placed in the system was to trap wear particles from the recirculating pump and valves located in the SPA.

The interface was highly effective at removing CO_2 and eliminated the interferences previously observed with direct-injection SFE/HPLC. Experimental FIA results (absorbance vs time) for a typical analysis of penicillin G in supercritical CO_2 are shown in Figure 5. The large vertical line at the beginning of the recording marks the point at which valve 1 was switched. Concentrations as low as 0.005 mg/mL were readily detected and quantified with UV detection. Data on the solubility (mg of solute/mL of supercritical CO_2 at the indicated pressure and temperature) of chloramphenicol and penicillin G at 40 °C as a function of CO_2 pressure are shown in Figure 6. A single data point was acquired at each pressure, and an apparent outlier at 4700 psi has been omitted from the data set. The results were in agreement with data previously obtained using a solvent trapping/off-line analysis approach. Of note is the sharp increase in solubility for penicillin G at pressures near 5000 psi. Significant increases

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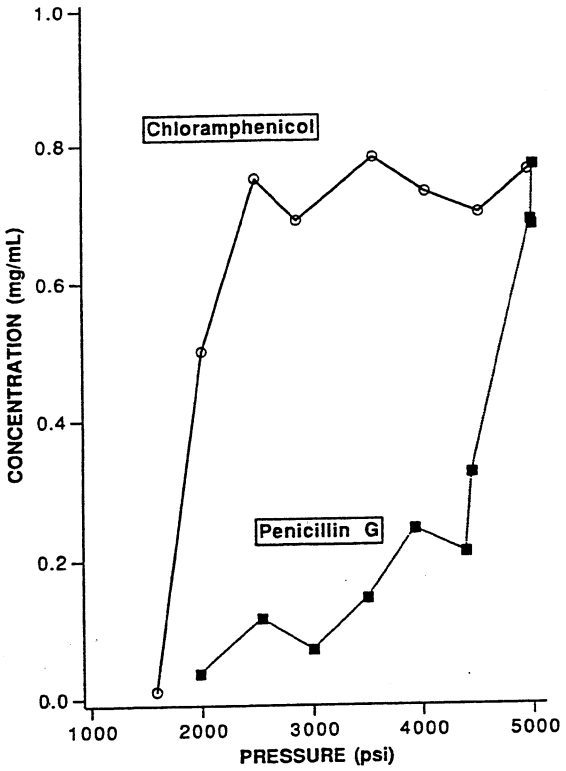

Figure 6. Solubility of chloramphenicol and penicillin G at 40 °C in supercritical carbon dioxide at various pressures: circles, chloramphenicol; squares, penicillin G.

in solubility in this pressure range have been noted for relatively polar veterinary antibiotics such as sulfamethazine and zoalene, and for less polar drugs such as monensin and salinomycin,[14] indicating the utility of high-pressure supercritical CO_2 as an extraction solvent for veterinary drugs.

Use of the membrane interface for on-line SFE/HPLC is expected to be straightforward. For this application, valve 2 (Figure 3) would be replaced by the high-pressure injection valve of the HPLC system, the detector would be eliminated from the interface system, and the upper connection of valve 1 would be directed to waste. An extract sample would be transferred into the sample loop of the HPLC injection valve using the procedure described for SFE/FIA, and this sample would then be injected into the HPLC system (not shown) for analysis. As the HPLC system would be completely isolated from the interface, constraints on carrier composition and flow rates necessitated by the phase separator would not limit the selection of HPLC conditions.

CONCLUSION

The membrane interface described here overcomes many of the deficiencies of previous designs for on-line coupling of SFE and liquid-phase analytical techniques. Interference from CO_2 is completely eliminated without the use of sorbents or other analyte-specific separation methods, providing quantitative transfer of analytes between the extraction and analytical systems. Rapid analysis of supercritical CO_2 extracts with an SFE/FIA system based on this interface has been demonstrated. By utilizing the membrane interface to transfer an extract sample to the injection valve of an HPLC system, an on-line SFE/HPLC system free of interference from CO_2 could be readily implemented. The membrane interface would also be useful for on-line monitoring of industrial supercritical extractions, such as decaffeination of coffee and extraction of oils.[15] On-line monitoring would permit extraction conditions to be adjusted in real time to accommodate variations in raw material characteristics and other process variables.

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